AP-MALDI-Q-IMS-TOF MS as a liquid sample mass profiling platform for food authenticity tests

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Oliver J. Hale [a] | Michael Morris [b] | Rainer Cramer [a]

a. Department of Chemistry, University of Reading, Whiteknights, Reading RG6 6AD, UK.
b. Waters Corporation, Stamford Avenue, Wilmslow SK9 4AX, UK

Overview

- **Development of workflows suitable for the analysis of crude liquid samples using a custom atmospheric pressure matrix-assisted laser desorption/ionisation (AP-MALDI) source.[1]**
- **High-throughput, real-time classification of samples with ion mobility-time-of-flight mass spectrometry (IMS-TOF MS) profiles is demonstrated.**
- **Further investigation and identification of individual compounds possible through offline data mining.**

Introduction

- MALDI is an established, sensitive ionisation technique, but requires crystalline samples for compatibility with its traditionally high-vacuum conditions. The persistent and stable ion beam generated from a glycerol-based liquid support matrix (LSM) at atmospheric pressure is more suitable for rapid, automated acquisition, as well as MS tuning and calibration. Sample longevity is high, enabling multiple analyses of the same droplet, with on-target storage at 4 °C previously demonstrated for many weeks.[2]
- Presented here are example workflows that take advantage of the AP-MALDI-Q-IMS-TOF MS platform (Figure 1) applied to the analysis of lipids extracted from milk.

Methods

- Lipids from samples of 6 milk types (bovine whole, bovine skimmed, caprine whole, ovine raw, ovine pasteurised and soya) were extracted with hexane/isopropanol (HIP; 3:2, v/v).
- Each liquid MALDI sample droplet consisted of 0.5 µL of HIP lipid extract (9:1, v/v) and 0.5 µL of LSM (2, 5-dihydroxybenzoic acid 25 mg/mL dissolved in H_2O/MeCN/glycerol, 3:7:6, v/v/v).
- 10-second analysis (30 Hz laser pulse repetition rate, 28 µJ/pulse) per sample generated IMS-TOF MS profiles in both positive and negative ion modes. The same droplets were used for both ion modes. TOF MS was run in 'sensitivity' mode.
- Online, real-time classification of milk types was achieved with Waters' Offline Model Builder (OMB) Recognition tool. TOF MS spectra from 45 samples per class, with an m/z range of 600-900, were used to build linear discriminant analysis (LDA) models (Figure 1A). In-depth analysis was performed with Progenesis QI and the EZInfo plugin (Figure 1B).

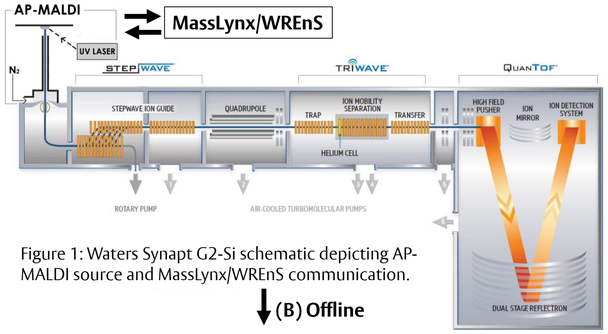

Figure 1: Waters Synapt G2-Si schematic depicting AP-MALDI source and MassLynx/WREnS communication.

(A) Online →

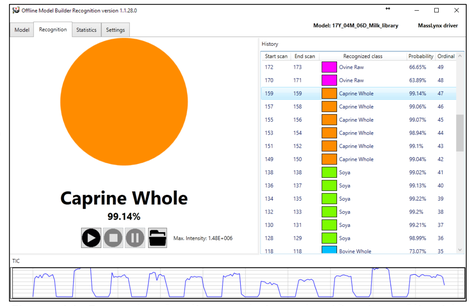

Figure 1A: Example of online, real-time classification of liquid MALDI samples using Waters' Offline Model Builder (OMB) Recognition software and an LDA model. The chromatogram beneath shows new sample acquisition where intensity rises.

(B) Offline ↓

Description	Adducts	Mass error (ppm)	CCS	ΔCCS (Å²)	Formula
PC 38:6 = G	M+H	2.40	298.00	4.55	$C_{46}H_{80}NO_8P$

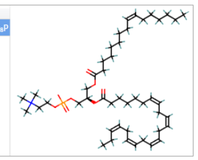

Figure 1B: Progenesis QI database-aided compound identification by accurate mass (tolerance <10 ppm) and CCS (tolerance <2.5%).

Results

- IMS was used to remove matrix-related chemical noise and enabled the calculation of collision cross-sections (CCS) for individual compounds when calibrated.
- CCS adds an extra dimension of information for assigning compound identity (Figures 1B and 2; Table 2).

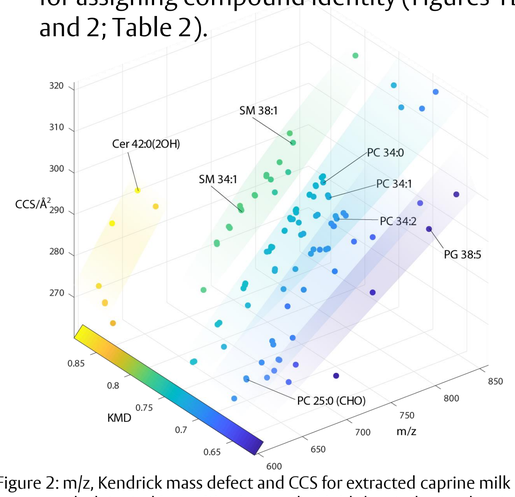

Figure 2: m/z, Kendrick mass defect and CCS for extracted caprine milk compounds detected in positive ion mode. Lipids located according to their classification: Cer = ceramides, SM = sphingomyelins, PC = phosphotidylcholines, PG = phosphotidylglycerols.

Table 1: Cross-validation results of LDA (A) and PLS-DA (B) models:

Model	MS Polarity	Correct classification rate %
LDA 4-class	+	100.0
	-	90.8
LDA 6-class	+	72.5
	-	78.9
PLS-DA 6-class	+	94.4
	-	84.6
	+ and – (offline combination of MS data from consecutive runs)	98.4

- Correct classification was determined by samples falling within the class-bounding sphere.
- 100% correct classification was achieved for the positive mode 4-class (organism-only) model
- Correct classification rate was reduced by inclusion of classes with very similar mass profiles, e.g. pasteurised and raw sheep milk.
- The EZInfo plug-in provided additional tools for data manipulation, enabling the combination of positive and negative ion mode data, but requiring a larger time investment.
- Individual compounds were identified from the mass profiles. Continued development of CCS databases will benefit this technique as many compounds cannot yet be matched to literature values.
- Examples of the two most influential compounds identified from the PLS-DA 6-class positive mode model are shown in Table 2.

Table 2: The two most influential compounds from PLS-DA 6-class positive mode.

Suggested ID	m/z (accuracy/ppm)	CCS/Å² (% difference from literature[3])
PC 34:1	760.5830 (-2.8)	298 (+1%)
SM 34:1	703.5719 (-4.1)	294 (+1%)

- The content of binary milk mixtures was determined from orthogonal partial least squares discriminant analysis (OPLS-DA) in EZInfo by relating intergroup separation and %mass (Figure 3).

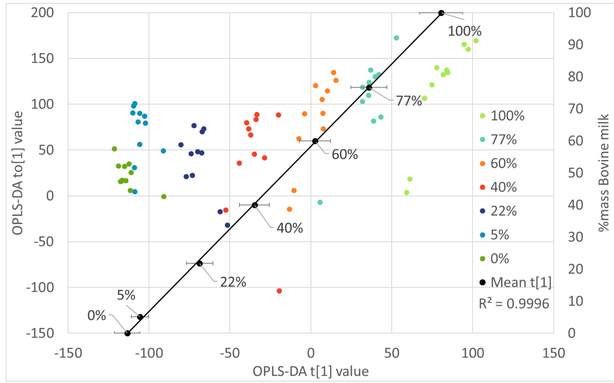

Figure 3: OPLS-DA plot of mixtures of bovine and caprine milk (left y-axis, coloured points) and the linear relationship between mean t[1] and %mass bovine milk (right y-axis, black points). Error bars show the standard deviation of each group.

Conclusions

- Liquid AP-MALDI-Q-IMS-TOF MS provides a good platform for high-throughput, real-time sample classification of milk samples.
- Offline processing enables deeper levels of analysis with existing software tools.
- Combination of positive and negative ion mode profiles has the potential to increase classification accuracy but is not possible online with TOF MS.

References

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Contact information

- Department of Chemistry, University of Reading, Whiteknights, RG6 6AD Reading, UK
- Email: o.j.hale@pgr.reading.ac.uk; r.k.cramer@reading.ac.uk
- http://www.reading.ac.uk/chemistry/about/staff/RKCGroupMembers.aspx